



# The ecosystem carbon sink implications of mountain forest expansion into abandoned grazing land: The role of subsoil and climatic factors

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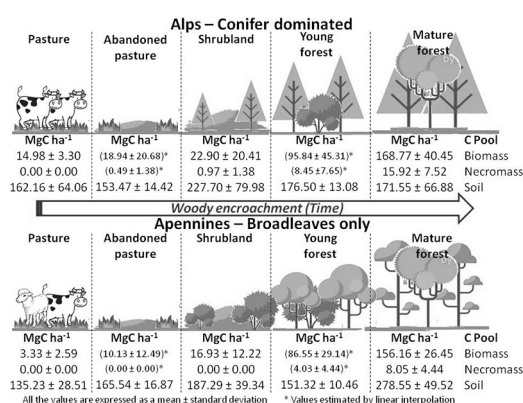
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## HIGHLIGHTS

- Woody encroachment over pasture can help mitigate the effects of climate change.
- Soil and biomass C pools were estimated in six areas with a chronosequence approach.
- Belowground C pools' (soil + below-ground biomass) changes lead ecosystem C dynamics.
- Winter air temperatures are the best predictors for sites' overall SOC stock changes.
- Subsoil omission leads to substantial ecosystem C underestimation.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Woody encroachment is a widespread phenomenon resulting from the abandonment of mountain agricultural and pastoral practices during the last century. As a result, forests have expanded, increasing biomass and necromass carbon (C) pools. However, the impact on soil organic carbon (SOC) is less clear. The main aim of this study was to investigate the effect of woody encroachment on SOC stocks and ecosystem C pools in six chronosequences located along the Italian peninsula, three in the Alps and three in the Apennines. Five stages along the chronosequences were identified in each site. Considering the topsoil (0–30 cm), subsoil (30 cm–bedrock) and whole soil profile, the temporal trend in SOC stocks was similar in all sites, with an initial increment and subsequent decrement in the intermediate phase. However, the final phase of the woody encroachment differed significantly between the Alps (mainly conifers) and the Apennines (broadleaf forests) sites, with a much more pronounced increment in the latter case. Compared to the previous pastures, after mature forest (>62 years old) establishment, SOC stocks increased by:  $2.1(\text{mean}) \pm 18.1(\text{sd})$  and  $50.1 \pm 25.2 \text{ Mg C ha}^{-1}$  in the topsoil,  $7.3 \pm 17.4$  and  $93.2 \pm 29.7 \text{ Mg C ha}^{-1}$  in the subsoil, and  $9.4 \pm 24.4$  and  $143.3 \pm 51.0 \text{ Mg C ha}^{-1}$  in the whole soil profile in Alps and Apennines, respectively. Changes in SOC stocks increased with mean annual air temperature and average minimum winter temperature, and were negatively correlated with the sum of summer precipitation. At the same time, all

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other C pools (biomass and necromass) increased by  $179.1 \pm 51.3$  and  $304.2 \pm 67.6 \text{ Mg C ha}^{-1}$  in the Alps and the Apennines sites, respectively. This study highlights the importance of considering both the subsoil, since deep soil layers contributed 38% to the observed variations in carbon stocks after land use change, and the possible repercussions for the carbon balance of large areas where forests are expanding, especially under pressing global warming scenarios.

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## 1. Introduction

Anthropogenic land-use change (LUC) is a key process greatly contributing to the observed changes in  $\text{CO}_2$  and other greenhouse gases (GHGs) in the atmosphere (Houghton, 2003; Kalnay and Cai, 2003). The contribution of LUC to observed changes in soil and vegetation carbon stocks is widely recognised (Foley et al., 2005), but its contribution to atmospheric  $\text{CO}_2$  sequestration and C storage is still a major uncertainty in the global carbon balance (Smith et al., 2014).

Human activities have transformed between one-third and one-half of the Earth land surface (Vitousek et al., 1997), particularly since the industrial revolution (Goldewijk and Battjes, 1997). Two main opposite processes are responsible for forest surface variation over time: deforestation and forest expansion (for both natural and human induced forces). According to FAO (2016), between 2010 and 2015, the world-wide forest surface has decreased at a rate of 3.3 million ha/yr, suggesting a net prevailing effect of deforestation. The same authors indicated the tropical ecosystems of South America, Southeast Asia and Africa as the main deforested zones in the last decades. On the opposite, a net forest increment has been described by FAO (2016) in boreal and temperate zones (including Mediterranean ones). Focusing at the European level, FAO (2016) reported a mean net forest surface increment of 0.4 million ha/yr (period 2010–2015). Zooming to Italy, Corona et al. (2012) and Marchetti et al. (2012) recorded a forest expansion of >500 kha (~1.7% of country area) between 1990 and 2008.

These results are in line with Archer (2010), who noticed that, after the Second World War, a substantial proportion of the global LUC has resulted from agricultural land abandonment. This trend has been widely observed in Europe (e.g. MacDonald et al., 2000; Tasser et al., 2007; Fuchs et al., 2013) and in Italy (Tasser and Tappeiner, 2002; Höchtl et al., 2005; Falcucci et al., 2007). Land abandonment in mountain territories, as a result of the movement of people in search of economic opportunities (Lambin et al., 2001), has led to widespread woody plant invasion on former pastures and croplands. As a consequence, new forests have generally established in less accessible and productive areas (Tappeiner et al., 2008; Zimmermann et al., 2010). Fuchs et al. (2013) estimated that, from 1950 to 2010, about 8% of the Southern Europe land had been transformed from grazing land to woody vegetation. In Italy, Corona et al. (2012) and Marchetti et al. (2012) have estimated that, from 1990 and 2008, pastures area has been reduced by about 1%.

Understanding the effect of woody encroachment on total ecosystem C stocks requires estimating all the C pools: soil organic carbon (SOC), above-ground biomass (AGB), below-ground biomass (BGB), woody debris and litter (Penman et al., 2003). Both human induced (e.g. plantations) and natural (e.g. natural forest expansion because of the reduction of human pressure) establishment of woody plant species lead to an increase in the structure and biomass of vegetation as well as in the amount of deadwood and litter and, in turn, to changes in SOC (Thuille and Schulze, 2006; Alberti et al., 2008; Guidi et al., 2014a, 2014b). While woody encroachment and afforestation/plantation over croplands often leads to a significant increase in SOC stocks (Post and Kwon, 2000; Guo and Gifford, 2002; Laganière et al., 2010; Poeplau and Don, 2013; Deng et al., 2016), grazing land afforestation generally decreases (e.g. Guo and Gifford, 2002; Thuille and Schulze, 2006; Li et al., 2012) or does not significantly affect SOC stocks (Laganière et al., 2010; Poeplau et al., 2011; Deng et al., 2016). Differences in SOC stock change results due to the latter LUC are, generally, linked to tree

species plantation, with a clearer SOC stock decrement under conifer with respect to broadleaves (Guo and Gifford, 2002). In addition, afforestation/plantation processes are generally characterised by a previous site preparation for new woody species allocation (Poeplau et al., 2011). According to Don et al. (2009), this practice encourages the soil organic matter mineralisation and causes the removal of grassland vegetation, leading, at least in the few years following the woody species plantation, to a decrease in SOC stock. On the other side, the SOC stock development for natural and spontaneous woody encroachment on pastures and grasslands, a process which is not characterised by the site preparation (Paul et al., 2002), is less clear (Poeplau et al., 2011; Guidi et al., 2014a). Indeed, recent studies show contrasting results, with negative (e.g. Alberti et al., 2008; Guidi et al., 2014a; Pinno and Wilson, 2011; La Mantia et al., 2013), positive (e.g. Feldpausch et al., 2004; Fonseca et al., 2011; La Mantia et al., 2013; Chiti et al., 2016) or statistically non-significant effects (Risch et al., 2008; La Mantia et al., 2013) of woody encroachment on SOC stocks. According to Jackson et al. (2002) and Alberti et al. (2011), these differences in SOC stock changes over time among sites are driven by study areas' climatic conditions, in particular by the mean annual precipitation.

In the Italian peninsula, most of the studies dealing with woody encroachment have been carried out in the Alps (e.g. Alberti et al., 2008; Risch et al., 2008; Guidi et al., 2014a), where generally mean annual precipitation is higher than the 1000 mm  $\text{yr}^{-1}$  threshold and where a summer dry stress does not generally occur. However, regions with more typically Mediterranean climatic conditions such as the Apennines, which are characterised by lower mean annual precipitations values and ordinary summer dry stress occurrence, have also undergone considerable woody encroachment. These areas are much less studied than Alps.

To accurately describe the changes occurring in the different C pools with woody encroachment on former pastures in Italy, we used a chronosequence approach (Walker et al., 2010) in six different areas located in the Alps and along the Apennines mountain ranges. Specific aims were: (i) to investigate the effect of woody encroachment on SOC stocks considering the whole soil profile (top and subsoil); (ii) to investigate the effect of climate on SOC stock changes in Mediterranean conditions and (iii) to determine the effect of woody encroachment on whole ecosystem C stocks (including all the pools listed in the IPCC). Particular attention was given to the transient dynamics between the endpoints of pasture and mature forest succession stages, for which no information is currently available in central and Southern Italian mainland. We hypothesised that: (i) since shrubs and trees develop much deeper roots than grass species, the subsoil SOC stock is fundamental to accurately quantify potential changes in soil carbon pool, (ii) precipitation and temperature are the main determinants of productivity in mountain areas, and thus of changes in soil and ecosystem carbon stocks, and (iii) the effect of woody encroachment differs between conifer and broadleaf forests not only because of plant species but also because of local climatic conditions.

## 2. Materials and methods

### 2.1. Study sites and chronosequence approach

The woody encroachment process was examined in two study areas: Alps and Apennines. Three sites each were selected along a longitudinal (or East-West) gradient in the Alps and along a latitudinal (or North-

South) gradient in the Apennines (Fig. 1), corresponding to the main orientation of the two mountain ridges. The sites differ in bedrock, soil type, climate and forest vegetation characteristics (Table 1). Monthly climatic data (average of minimum air temperature, average of mean air temperature, average of maximum air temperature and precipitation) for each site for the period 1951–2015 were provided by Bologna ISAC-CNR who estimated them using the methodology of Brunetti et al. (2012).

In addition to mean annual precipitation (MAP) and mean annual temperature (MAT), we calculated winter cold ( $T_{min_w}$ ) and summer dry stress ( $Ps$ ) derived from Mitrakos (1980), and the potential net primary productivity ( $potNPP$ ):

- $T_{min_w}$ , which is the average of minimum winter temperatures of the three coldest months (December, January and February);

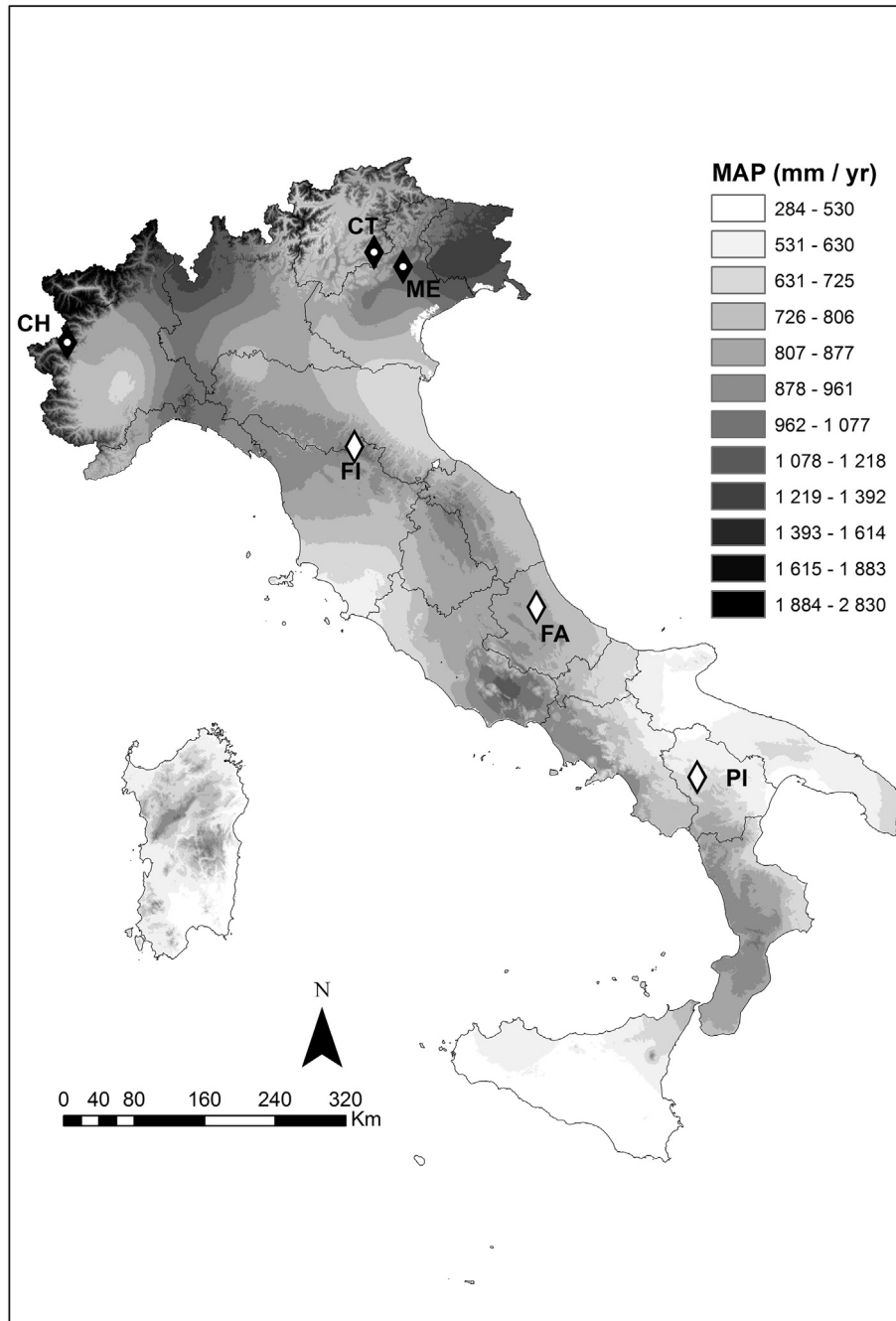
- $Ps$ , which is the sum of averaged summer precipitation in the three summer months (June, July and August);
- The Lieth (1972) potential net primary productivity ( $potNPP$ ) estimated on the base of the limiting factor of MAP or MAT (Eq. (1)), as

$$potNPP = \min(NPPt, NPPp) \quad (1)$$

where

$$NPPt = 3000 \times (1 + \exp(1.315 - 0.119 \times MAT))^{-1} \quad (2)$$

$$NPPp = 3000 \times (1 - \exp(-0.000664 \times MAP)) \quad (3)$$



**Fig. 1.** Selected sites located along the Italian peninsula. Filled symbols represent Alps sites (mainly conifers), while Apennines ones (broadleaves only) are represented by empty symbols. The Alps sites are Castello Tesino (CT), Mel (ME) and Chianocco (CH). The Apennine sites are Firenzuola (FI), Farindola (FA) and Pignola (PI) according to the sites' abbreviation described in Table 1. The background grey scale summarises the Italian mean annual precipitation gradient from dry (pale colours) to wet areas (dark colours).

**Table 1**  
List of biophysical characteristics of each selected site.

Variable	Site					
	Castello Tesino	Mel	Chianocco	Firenzuola	Farindola	Pignola
Site abbreviation	CT	ME	CH	FI	FA	PI
Lon./Lat. (WGS84)	11.650/46.125	12.071/45.969	7.202/45.177	11.320/44.140	13.783/42.433	15.819/40.583
Study area	Alps	Alps	Alps	Apennines	Apennines	Apennines
Bedrock	Calcareous	Calcareous	Calcareous	Chaotic	Calcareous	Calcareous
Soil type (IUSS Working Group WRB, 2015)	Phaeozems	Cambisols	Cambisols	Cambisols	Phaeozems	Phaeozems
Forest type	Conifers	Conifers	Mix (mainly conifer)	Broadleaves	Broadleaves	Broadleaves
MAP (mm) <sup>1</sup>	1286	1670	967	1620	1136	957
MAT (°C) <sup>2</sup>	4.6	6.5	8.4	10.1	9.8	11
Tmin <sub>w</sub> (°C) <sup>3</sup>	−6.5	−5.3	−3.4	−0.4	−1.4	0.9
Ps (mm) <sup>4</sup>	403.1	422.5	249.5	183.4	204.9	104.8
potNPP (g <sub>dm</sub> m <sup>−2</sup> yr <sup>−1</sup> ) <sup>5</sup>	951.0	1103.5	1265.4	1415.3	1388.6	1410.9

<sup>1</sup> MAP = Mean Annual Precipitation.

<sup>2</sup> MAT = Mean Annual Temperature.

<sup>3</sup> Tmin<sub>w</sub> = Average of minimum winter temperature of the three coolest months (December, January and February).

<sup>4</sup> Ps = Average of the sum of the summer precipitation in the three months June, July and August.

<sup>5</sup> potNPP = potential net primary productivity estimated according to Lieth (1972). This variable is expressed in grams of dry matter per square metre per year.

The variable *potNPP* is expressed in grams of dry matter per square metre per year.

Site selection was based on the information collected from local research groups, forest rangers, available literature and local population. The validity of the previous information was verified by comparison with airborne digital orthophoto series for the last 60–70 years at each site. The most representative images of each site were downloaded by Geoportale Nazionale website (MATTM, 2015) or bought from the Istituto Geografico Militare (IGM, 2014) and analysed by visual interpretation on a GIS. This process allowed the application of the chronosequence approach based on the *space-for-time* substitution concept (Austin, 1981; Huggett, 1998; Walker et al., 2010). This consists on a synchronic (i.e. at the same time) analysis of a series of spatially separated areas, representing the different succession stages of the considered woody encroachment process. Depending on the site, five or six succession stages per chronosequence were identified: two extreme succession stages (pasture T0; and mature forest T5) which had not shown any substantial variation in vegetation cover during the time span covered by the orthophoto series; and three or four intermediate succession stages (T1, T2, T3 when present, and T4). These stages were characterised by a grazing land cover at the beginning of the considered period (in the oldest orthophoto) and had been encroached by woody plant species in different moments in the past. In particular, at the present time:

- T1 succession stages are characterised by a predominant grass vegetation cover with only some woody plants species (mean of 12.5 years from pastures abandonment);
- T2 succession stages are characterised by a more dense shrubs vegetation with respect to T1 and perennial grasses (mean of 24 years from pastures abandonment);
- T3 succession stages are characterised by a young forest vegetation sometime mixed to underlying shrubs coverage (mean of 30 years from pastures abandonment);
- T4 succession stages are characterised by adult tree species with or without underlying shrubs stratum (mean of 52 years from pastures abandonment).

Because of the extreme medium- and small-scale landscape heterogeneity of the mountain territories where the woody encroachment takes place in Italy, the succession stages unavoidably showed some variability in slope position, exposure, steepness and elevation (Table 2). All these parameters were recorded and the exposure data were broken down into sine and cosine of the original aspect angle in order to consider both the variability along East–West and North–

South axes, respectively. The variations of all these parameters were minimised by selecting all the succession stages from each chronosequence in a close-range area, no >2 km apart from each other, and excluding zones with significant variation in lithology, pedology and phytoclimate. In addition, all the sources of variability listed above have been taken into account as a potential source of bias. Finally, the succession stages were described on the basis of their woody plant species (Table 2).

## 2.2. Soil sampling and processing

The soil sampling design followed the protocol proposed by the Joint Research Centre of the European Commission (Stolbovoy et al., 2007). In each succession stage, three squared cells were selected with a pseudo-random sampling approach, satisfying the original cells non-contiguity constraint. Although the original method is based on the guidelines recommended by Penman et al. (2003), some modifications were adopted. In particular, we considered the whole soil profile reaching the bedrock depth: 0–5, 5–15, 15–30 (topsoil), and 30–50, 50–70 cm (subsoil), depth intervals.

In each sampling cell, soil samples were collected following a grid of 5 × 5 sampling points. For each layer, a homogeneous composite sample ( $n = 25$ ) was collected and transported to the laboratory for physico-chemical analyses. In the central point of each sampling plot a small profile was dug down to 30 cm depth to describe the topsoil horizons according to Schoeneberger et al. (2002). Samples for bulk density (BD) estimation were collected from the three upper layers (0–5, 5–15, 15–30 cm) using a metal cylinder with a known volume (diameter = 5 cm, height = 5 cm, vol. = 34.3 cm<sup>3</sup>) according to Blake (1965). No BD samples were collected from the layers below 30 cm depth because of the excess of the rock fragments in the subsoil. For these layers, BD was estimated using the pedotransfer function (Eq. (4)) proposed by Adams (1973), which showed the best performance according to the review of De Vos et al. (2005).

$$BD = \frac{1}{(a + b \times \%C)} \quad (4)$$

where BD is the soil bulk density (kg m<sup>−3</sup>),  $a$  and  $b$  are constants, and %C is the soil C percentage. We decided to applied the values of  $a = 0.686$  and  $b = 0.085$  suggested by Chiti et al. (2012) for the 30–100 cm soil compartment because BD estimations with the coefficients used by the authors for the 0–30 cm soil compartment fitted very well with our topsoil BD measurements.



**Table 2**

Summary of the main succession stage characteristics for each site. Woody plant species nomenclature follows The Plant List (2013).

Site	Succession stage	Age <sup>1</sup>	Elevation (m.a.s.l.)	Exposure	Steepness (%)	Position <sup>2</sup>	Land Use	Soil pH	Woody plant species
CT	T0	0	1700	S	26.24	Footslope	Pasture	5.0	None
CT	T1	9	1650	SE	48.85	Backslope	Pasture	6.8	Trees: <i>Picea abies</i> (L.) H.Karst.
CT	T2	18.5	1900	S	62.82	Backslope	Unmanaged shrubland	4.9	Trees: <i>P. abies</i> , <i>Larix deciduas</i> Mill.; shrubs: <i>Rhododendron</i> sp., <i>Calluna vulgaris</i> (L.) Hull, <i>Junioperus communis</i> var. <i>Saxatilis</i> Pall.
CT	T3	28	1450	SE	27.9	Footslope	Unmanaged forest	5.5	trees: <i>P. abies</i>
CT	T4	51.5	1750	S	64.13	Backslope	Unmanaged forest	6.4	Trees: <i>P. abies</i> , <i>L. decidua</i>
CT	T5	>62	1750	SE	71.01	Backslope	Unmanaged forest	5.7	Trees: <i>P. abies</i> , <i>L. decidua</i>
ME	T0	0	1325	N	31.6	Shoulder	Pasture	4.8	None
ME	T1	5	1270	N	29.97	Shoulder	Unmanaged grassland	4.7	Shrubs: <i>Rubus idaeus</i> L.
ME	T2	29	1260	W	53.07	Backslope	Pasture and shrubland	5.2	Trees: <i>P. abies</i> ; shrubs: <i>Rubus idaeus</i> L., <i>J. communis</i> , <i>Corylus avellana</i> L., <i>Sorbus aria</i> (L.) Cranz
ME	T3								
ME	T4	40	1250	N	38.63	Backslope	Forest plantation	4.3	Trees: <i>P. abies</i> , shrubs: <i>Vaccinium myrtillus</i> L.
ME	T5	>62	1225	NW	29.21	Backslope	Forest plantation	4.1	Trees: <i>P. abies</i>
CH	T0	0	1200	SW	37.35	Backslope	Grassland	6.5	None
CH	T1	12	1200	SW	47.49	Backslope	Unmanaged shrubland	6.3	Shrubs: <i>Rubus ulmifolius</i> Schott
CH	T2	22	920	W	75.65	Backslope	Unmanaged shrubland	6.9	Trees: <i>Tilia cordata</i> Mill., <i>Fraxinus excelsior</i> L., <i>Quercus pubescens</i> Willd., <i>C. avellana</i> , <i>Laburnum alpinum</i> (Mill.) Bercht. & J.Presl, <i>Pinus sylvestris</i> L., <i>Acer pseudoplatanus</i> L., <i>J. communis</i> , <i>Prunus avium</i> (L.) L.
CH	T3								
CH	T4	42.5	110	SW	40.96	Backslope	Unmanaged forest	6.2	Trees: <i>Fagus sylvatica</i> L., <i>P. sylvestris</i>
CH	T5	>62	110	W	46.89	Backslope	Unmanaged forest	5.6	Trees: <i>F. sylvatica</i> , <i>P. sylvestris</i>
FI	T0	0	875	SE	15.03	Backslope	Pasture	7.1	None
FI	T1	19	900	SE	20.23	Backslope	Pasture and shrubland	7.4	Shrubs: <i>Rosa canina</i> L., <i>J. communis</i>
FI	T2	25	925	SW	16.28	Backslope	Pasture and shrubland	7.1	Trees: <i>Pyrus communis</i> L.; shrubs: <i>Prunus spinosa</i> L., <i>Crataegus monogyna</i> Jacq., <i>R. canina</i> , <i>J. communis</i> , <i>Ligustrum vulgare</i> L.
FI	T3	32.5	860	SE	37.34	Backslope	Unmanaged forest	7.0	Trees: mix broadleaves; shrubs: <i>P. spinosa</i> , <i>C. monogyna</i> , <i>R. canina</i> , <i>J. communis</i> , <i>L. vulgare</i>
FI	T4	64	850	S	17.44	Backslope	Unmanaged forest	6.8	Trees: mix broadleaves with <i>Quercus cerris</i> L., <i>Q. pubescens</i> ; shrubs: <i>P. spinosa</i> , <i>C. monogyna</i> , <i>R. canina</i> , <i>J. communis</i> , <i>L. vulgare</i>
FI	T5	>68	925	SE	26.07	Backslope	Unmanaged forest	6.7	Trees: <i>Q. cerris</i> , <i>Acer campestre</i> L., <i>P. communis</i> , <i>Ulmus minor</i> Mill., <i>A. campestre</i> , <i>Fraxinus ornus</i> L., <i>Acer opalus</i> Mill.; shrubs: <i>C. monogyna</i> , <i>L. vulgare</i>
FA	T0	0	1140	SW	19.52	Backslope	Pasture	6.4	None
FA	T1	12	1160	S	28.29	Backslope	Pasture and shrubland	7.1	Shrubs: <i>R. canina</i> , <i>P. spinosa</i>
FA	T2	24	1050	S	27.94	Backslope	Unmanaged shrubland	6.3	Trees: <i>A. campestre</i> , <i>P. communis</i> ; shrubs: <i>R. canina</i> , <i>C. monogyna</i> , <i>R. ulmifolius</i> , <i>P. spinosa</i>
FA	T3								
FA	T4	65.5	1190	SW	39.48	Shoulder	Tracked forest	6.7	<i>F. sylvatica</i>
FA	T5	>70	1270	E	32.64	Backslope	Unmanaged forest	6.4	<i>F. sylvatica</i>
PI	T0	0	1000	E	20.15	Backslope	Pasture	7.3	None
PI	T1	18	980	N	18.1	Backslope	Pasture and shrubland	6.6	Shrubs: <i>C. monogyna</i> , <i>P. spinosa</i> , <i>R. canina</i> , <i>R. ulmifolius</i>
PI	T2	24.5	1000	N	16.7	Backslope	Pasture and shrubland	7.1	Shrubs: <i>C. monogyna</i> , <i>P. spinosa</i> , <i>R. canina</i> , <i>Spartium junceum</i> L., <i>Lonicera caprifolium</i> L., <i>R. ulmifolius</i> and <i>P. communis</i>
PI	T3								
PI	T4	50.5	1030	N	22.26	Backslope	Tracked forest	6.1	Trees: <i>q. cerris</i>
PI	T5	>60	1200	N	37.53	Backslope	Managed forest	6.0	Trees: <i>q. cerris</i> ; shrubs: <i>ilex aquifolium</i> L.

<sup>1</sup> Age column represents the time since the woody encroachment started and is measured in years before present. The intermediate date between the two subsequent airborne images (of each site photograph series) showing interpretable differences in the canopy cover was considered as the date of secondary succession start for each intermediate succession stage.

<sup>2</sup> Position parameter refers to the location of each succession stage along the mountains slope.

All the soil samples were dried out in an oven at 60 °C until they reached a constant mass. The dry samples for physico-chemical analyses were crushed and sieved at 2 mm. Both the coarse rock fragments (>2 mm) and the fine earth (<2 mm) fractions were weighed to estimate the percentage of each component.

The fine earth (i.e. the soil) pH was potentiometrically measured in deionised water with a soil–liquid ratio of 1:2.5 (Van Reeuwijk, 2002), with the Mettler Toledo Easy pH Titrator System. To determine SOC concentration, aliquots of each fine earth sample were pulverised to a soil dust and weighted with a precision scale ( $\pm 1 \mu\text{g}$ ). Then, they were

treated two consecutive times with 40 µl of 10% HCl solution to remove inorganic carbonates. The organic C ( $C_{org}$ ) was measured by dry combustion using a CHN-Elemental Analyser (Thermo-Finnigan Flash EA112 CHN).

Finally, the  $SOC_{stock}$  ( $kg\ C\ m^{-2}$ ) was determined for each layer according to Boone et al. (1999) Eq. (5) as:

$$SOC_{stock} = C_{org} \times BD \times d \times \left[ 1 - \left( \frac{\%mass_r}{100} \right) \right] \quad (5)$$

where  $C_{org}$  is the organic C concentration ( $kg\ C \cdot kg^{-1}$  soil), BD is the bulk density (in  $kg\ soil\ m^{-3}$ ),  $d$  is the depth of the considered layer (cm) and  $\%mass_r$  is the percentage in mass of rock fragments.

In addition, for each soil profile, the SOC stocks of each layer were added together in order to estimate topsoil (0–30 cm), subsoil (30–70 cm) and whole profile (0–70 cm) SOC stocks. The SOC stocks of different soil profiles were compared on the basis of the equivalent soil depth approach. This approach was preferred to the equivalent soil mass method suggested by Ellert and Bettany (1995) for two main reasons: (1) the Stolbovoy et al. (2007) sampling design used in this study is based on the equivalent soil depth approach; and (2) we assume that root systems affect biopores formation and thus the soil bulk density. Therefore, in our case, the possible differences in soils bulk density had to be attributed to the root system growth rather than to the result of agricultural tillage practices (as described by Ellert and Bettany, 1995).

### 2.3. Living biomass estimation

AGB and dead organic matter were estimated in three succession stages per chronosequence: T0 (pasture), T2 (shrubland) and T5 (mature forest). In each of them, we selected three circular sampling plots (13 m of radius), around the profiles used for soil description, following the Italian national forest inventory protocol of the Italian Ministry of Agricultural and Forestry Policies (MPAF, 2006) and Bovio et al. (2014). The original sampling protocol version was applied for pastures and mature forests, while it was slightly modified for the shrubland succession stages. Indeed, the high shrubs spatial heterogeneity in T2 succession stages forced us to estimate their biomass in the whole circular sampling plots of 13 m radius, instead of the smaller 2 m radius areas as described in the original sampling protocol (see Table S3 and Fig. S8). Trees AGB was estimated according to allometric equations suggested by Tabacchi et al. (2011). The same approach was adopted for *Corylus avellana* L. and *Rosa canina* L., which were the only two shrub species we found in the field with published allometric equations in the consulted literature (Alberti et al., 2008; Blujdea et al., 2012). For the other shrub species, all the individuals of each species were counted in each sampling area. Only one representative (in terms of diameter at collar height, total plant height, and the two crown diameters dimensions) plant per species was collected in the field and transported to the laboratory for the dry mass procedure. The same approach was adopted for all grass species.

Because of the lack of specific root-to-shoot ratios (R/S) for all the woody and grasses species observed, the BGB was estimated by adopting the vegetation-specific R/S according to Mokany et al. (2006).

### 2.4. Dead organic matter estimation

For dead organic matter estimation, we followed the sampling protocol by Alberti et al. (2008) summarised in Table S3 and Fig. S8. Dead organic matter was divided into litter, fine (FWD;  $\emptyset < 5$  cm) and coarse woody debris (CWD;  $\emptyset > 5$  cm) on the forest floor, and standing dead trees. The litter layer was collected in the succession stages where it was present using a  $20 \times 20$  cm plot randomly placed inside each sampling plot. FWD was collected in four sampling areas of  $0.25\ m^2$  per plot. Litter and FWD mass was estimated drying out and weighting the

collected samples in the laboratory. For the CWD, because no samples were collected to estimate wood density data, it was only possible to estimate the volume ( $V_{CWD}$  in  $m^3\ ha^{-1}$ ) according to the methods (Eq. (6)) of Harmon and Sexton (1996).

$$V_{CWD} = 9.869 \times \sum \left( \frac{d^2}{8L} \right) \quad (6)$$

where  $d$  is the fragment diameter (m) and  $L$  (m) is the sum of the lengths of both North-to-South and East-to-West 26 m-long transects.

The volume of standing dead trees ( $V_{SDT}$  in  $m^3$ ) was estimated using the formula (Eq. (7)) suggested by Alberti et al. (2008):

$$V_{SDT} = 0.5 \times \left( \frac{\pi}{4} \right) \times DBH^2 \times H \quad (7)$$

where  $DBH$  (cm) and  $H$  (m) are diameter at breast height and total height, respectively.

Then, the measurements were converted into mass by means of a wood density estimation measured by species-specific fresh wood density (Global Wood Density Database, 2015) and the decrease decay wood density class values described by Alberti et al. (2008).

Finally, all living biomass and dead organic matter pools were converted to carbon, adopting the 0.475 conversion factor proposed by Magnussen and Reed (2004).

## 3. Calculation and statistical analyses

Considering the soil C pool, the prevalent role of the succession stage in explaining the SOC stock changes with respect to the other variables (elevation, steepness, exposure and slope position) was tested in all the sites together and separately for each of the two study areas, by means of a linear mixed-effect model. Then, the values of each continuous explanatory variable were feature-scaling standardised (normalised) on the basis of the variability inside each site. Finally, succession stage, slope position, elevation, steepness, North and East exposures were considered as fixed-effect of the saturated model, while site was considered as random (intercept). The saturated model was progressively simplified removing the variables that did not significantly affect the SOC stock changes. The simplest model that does not statistically differ from the saturated one (ANOVA likelihood comparison with  $P = 0.05$ ) was preferred. The weight of the selected variables was evaluated removing one by one in the simplest model and comparing the associated Akaike Information Criterion (AIC) values. For the simplest model, residuals homoskedasticity and normality assumptions were verified by means of residuals vs. fitted plot, quantile-quantile plot and by means of Shapiro-Wilk normality test. Each of these analyses was performed three times: one for the whole soil profile (0 cm – bedrock), one for the topsoil (0–30 cm depth) and one for the subsoil (30 cm – bedrock).

The difference in proportional SOC stock change from pasture to forest (i.e. forest SOC stock/pasture SOC stock) among the study areas was tested by a non-parametric Kruskal-Wallis comparison, both for the topsoil and subsoil, as a previous test on the residuals showed that they were not normally distributed.

SOC concentration changes along the three main chronosequence succession stages (pasture [T0], shrubland [T2] and mature forest [T5]) for both soil profiles were tested by a two-way ANOVA. The following pairwise  $t$ -test comparison was performed when necessary. These analyses were performed separately for each study area.

The proportional SOC stock change from pasture to forest in each chronosequence was plotted against the values of the climatic indexes (see Table 1). A linear model was applied to the data in order to explain the relationship between SOC stock changes and climatic predictors. No normality assumption was made because of the limited number of sites ( $n = 6$ ) considered in the present study.

Finally, each C pool was considered separately. The mean C stocks were compared among succession stages and study areas, by means of two-way ANOVAs. The following pairwise *t*-test comparison was performed when necessary. The same analyses were performed considering the whole ecosystem C stock (i.e., the sum of all the stock of each pool).

All the statistical analyses were performed in RStudio (RStudio Team, 2015). Linear-mixed models, Kruskal-Wallis comparison, linear regressions and two-way ANOVAs were performed by means of the following functions, respectively: *lmer* contained in the R package *lme4* (Bates et al., 2017), *kruskal.test*, *lm*, *anova(lm)*.

## 4. Results

### 4.1. Effect of woody encroachment on SOC stocks

The simplest statistical model included only the following variables: the succession stage, the exposure along North-to-South axis, and the interaction between succession stages and study areas (Table S4); the “site” was instead treated as a random intercept. High significant differences between the simplest model without the succession stage factor and the corresponding model without the exposure one were confirmed by the ANOVA statistics ( $\chi^2(9) = 86.71$ ,  $P < 0.001$ ). In addition, the higher AIC of the simplest model without the succession stage factor with respect to that of the simplest model without the exposure factor indicated that the succession stage was more important than the exposure in explaining the SOC stock changes over time in both study areas.

In the Alps sites (Fig. 2a), only the shrubland succession stage [T2] showed a significantly higher SOC stock ( $+65.5 \pm 13.4 \text{ Mg C ha}^{-1}$ ;  $P < 0.001$ ) with respect to the pasture succession stage [T0]. In the Apennines (Fig. 2b), significantly higher SOC stock with respect to pasture succession stage [T0] were estimated in the first encroached succession stage [T1] ( $P < 0.01$ ), in shrubland [T2] ( $P < 0.001$ ) and in the mature forest [T5] ( $P < 0.001$ ) (Table S4).

The simplest model outputs showed that woody encroachment in the Alps sites affected SOC stocks differently than in the Apennines sites. Indeed, highly significant differences ( $P < 0.001$ ) were observed when comparing Alps and Apennines mature forest succession stages, with higher SOC stock values observed in the latter one (Fig. 2, Table S4).

For the topsoil, the comparison between Alps and Apennines sites showed considerable differences only for the first succession stage [T1] ( $P = 0.076$ ) and mature forest [T5] ( $P < 0.001$ ), with higher SOC stock in the Apennines than in the Alps (Table S5). While in the Alps sites there were no significant differences between succession stages ( $P = 0.068$ ), in the Apennines, higher SOC stock than in pasture [T0] were estimated for the first succession stage [T1] ( $P = 0.04$ ), shrubland [T2] ( $P = 0.005$ ) mature forest [T5] ( $P < 0.001$ ) (Table S5). Similarly to the whole profile, high significant differences between the simplest model without the succession stage factor and the corresponding model without the exposure factor have been estimated ( $\chi^2(9) = 33.38$ ,  $P < 0.001$ ) with a higher AIC estimator in the former case (AIC of 898.3) than in the latter one (AIC of 882.9).

In the subsoil, the comparison between Alps and Apennines sites showed significant differences only for the mature forest succession stage [T5] ( $P < 0.001$ ), with significantly higher SOC stocks in the Apennines than in the Alps. In both, the Alps and Apennines sites, the shrubland [T2] had significantly higher (Alps:  $P < 0.001$ , Apennines:  $P < 0.001$ ) SOC stock than the initial pasture [T0] sites. However, in the subsoil of Apennine sites, significantly higher values in SOC stocks with respect to initial pasture [T0] were estimated for the first succession stage [T1] ( $P = 0.025$ ) and mature forest [T5] ( $P < 0.001$ ) in the subsoil (Table S6).

When differences in SOC stocks between succession stages were significant for the whole soil profile, it was mainly attributable to subsoil SOC stock changes (Fig. 3). Indeed, the proportional changes in the SOC stocks from pasture to mature forest were significantly different

between sites located in the Alps (mature forest SOC stock/pasture SOC stock = 1.06 and 1.11) and those in the Apennines (2.06 and 3.66) for the whole soil profile and the subsoil compartment, respectively. No significant differences were observed for the topsoil.

### 4.2. Changes in SOC through the soil profile

Considering the three main chronosequence stages (pasture, shrubland and mature forest), in each of them the SOC concentrations progressively decreased through the soil profiles (Fig. 4; lower case letters describe statistically significant differences in each panel). This trend can be observed in both Alps and Apennines sites groups. In the Alps, no significant differences were observed among succession stages (Fig. 4; capital letters denote significant differences between stages in each column). However, shrublands had consistent, although not highly significant, higher C contents with respect to both pastures ( $P = 0.053$ ) and to mature forest stages ( $P = 0.114$ ). In the Apennines group, mature forests had significantly higher C contents with respect to both pastures ( $P = 0.003$ ) and mature forest stages ( $P = 0.014$ ).

### 4.3. The role of climate on the effect of woody encroachment on SOC stocks

SOC stock increased with mean annual temperature (MAT) (Fig. 5a). In addition, SOC stock increased with increasing average of minimum winter temperature ( $T_{min,w}$ ) (Fig. 5b), and decreased with increasing sum of the summer precipitation (Ps) (Fig. 5c). In all cases the slope of the linear regressions is significantly different from zero ( $P < 0.05$ ) and with a goodness-of-fit ( $r^2$ ) higher than 0.65. Even if SOC stock is significantly correlated with both  $T_{min,w}$  and Ps, our results show that  $T_{min,w}$  explains 10% more variability than Ps. Neither MAP nor other climatic variables statistically affected SOC stock changes.

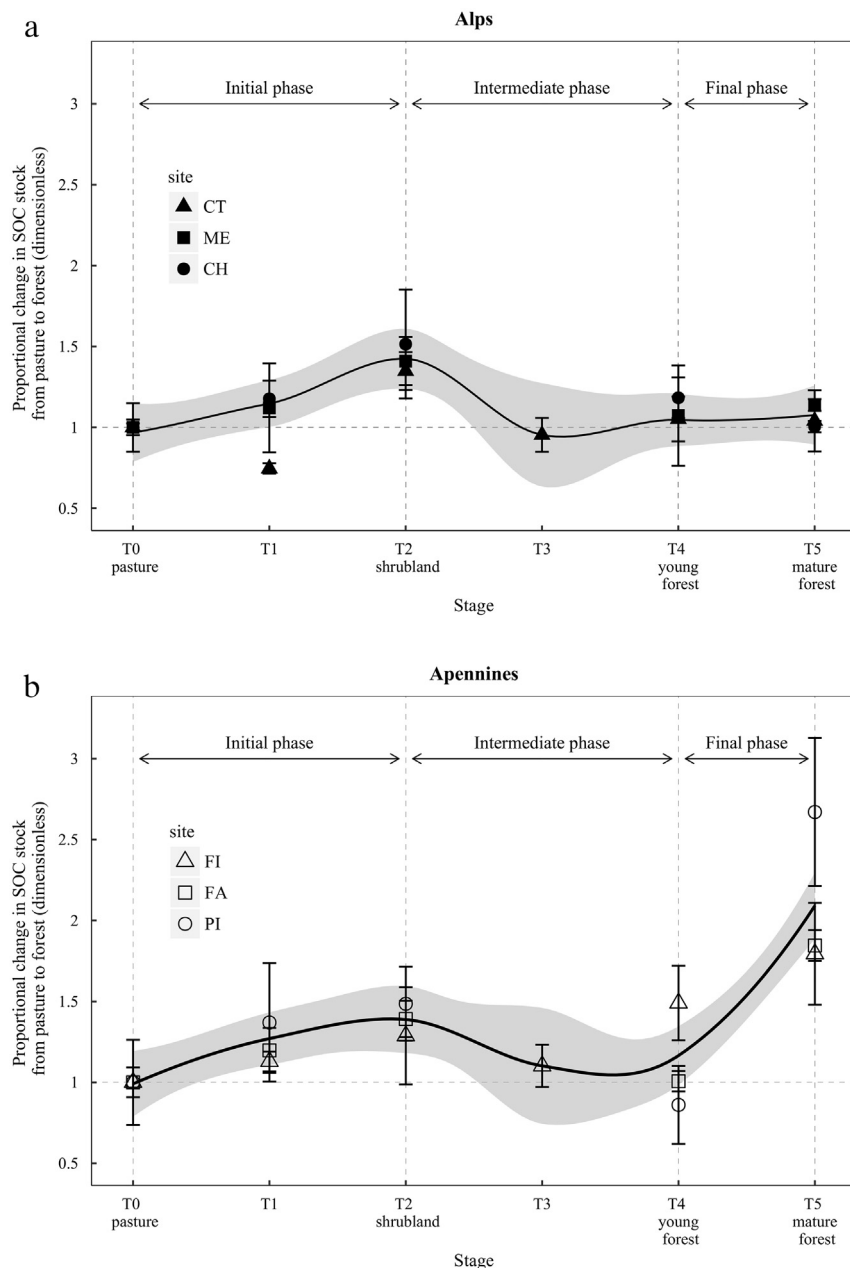
### 4.4. Effect of woody encroachment on ecosystem C stocks

While a significant difference (increment) appeared only in SOC stock between pasture [T0] and shrubland [T2], above-ground living biomass, woody debris, litter and below-ground living biomass C stocks increased over time with a significant difference ( $P < 0.05$ ) between mature forest stage [T5] and the previous ones (pasture [T0] and shrubland [T2], see Fig. 6). Woody encroachment had similar effects in the Alps and Apennines for all the C pools and all the succession stages, with the exception of litter and SOC stocks in the mature forest. Indeed, Alps mature forests were characterised by both significantly higher litter stocks and significantly lower SOC stocks compared to corresponding succession stages in the Apennines.

The ecosystem C stock estimated by adding all the C pools together showed a progressive and significant increase along the woody encroachment process. The post-hoc comparison revealed that significant differences were present between each pair of succession stages ( $P < 0.05$ ). No significant differences were observed between the sites in the Alps and in the Apennines (Fig. 7).

## 5. Discussion

This study showed that the most important parameter explaining SOC stock changes along the woody encroachment process was the time since abandonment (succession stage). Site exposure was also found to have a significant effect on SOC stock changes, in particular due to the variation along the North-to-South facing slopes, with an increase observed from North to South. This result is in line with Yimer et al. (2006) and Sigua and Coleman (2010), and we argue that the exposure can be considered as a proxy for other environmental parameters (like temperature and radiation) because it is linked to the higher forest potential net primary productivity (NPP) on warm South-exposed slopes with respect to the cooler North-exposed ones. This explanation is consistent with the insight obtained by applying the methodology of



**Fig. 2.** Whole profile SOC stock proportional changes with respect to the mean of previous pasture SOC stock for (a) sites with conifers (Alps), and (b) without conifers (Apennines). All the symbols represent mean values  $\pm$  standard deviations. The shaded areas represent the confidence intervals (mean  $\pm$  standard deviation) around the mean curves.

Lieth (1972), which identifies low temperature, rather than precipitation, as the main limiting factor for the vegetation potential NPP estimation of the sites considered (Pignola, the most southern one, being an exception).

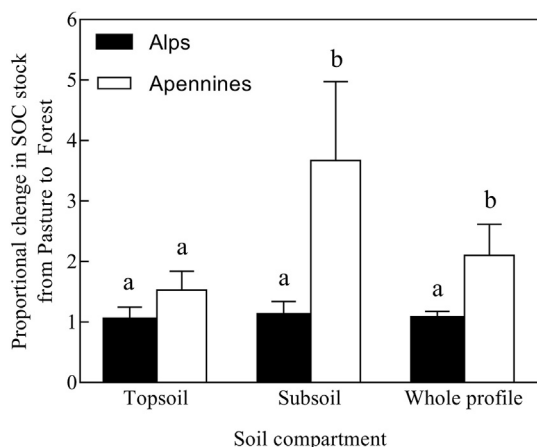
In addition, this study showed that the subsoil stores a significant percentage of the whole profile SOC stock (an average of 38%) and that the effect of woody encroachment on SOC stock changes are significant not only on the topsoil compartment, but also in the subsoil one.

### 5.1. Effect of woody encroachment on SOC stocks

Unlike previous studies (e.g. Montané et al., 2007; Alberti et al., 2008; Pinno and Wilson, 2011; Guidi et al., 2014a), non-monotonic SOC stocks trends were observed in all the study sites. Indeed, we observed a significant SOC stock increment in the initial phase of the process (from pasture to shrubland succession stages) followed by comparable decrease during the intermediate phase (between shrubland

and young forest succession stages). A similar trend was observed by La Mantia et al. (2013) in thermomediterranean climatic conditions, and by Thuille et al. (2000). The latter study, in the slightly different context of afforestation, observed a similar increase in the 25-year-old succession stage in one site in the Italian Alps. The authors attributed this increase to the extensive grazing, which could have added a significant amount of C to the soil through animal faeces. Although in the present study some grazing pressure was observed in the intermediate succession stage of some sites (e.g. Castello Tesino and Mel), it cannot be considered extensive. However, we observed that intermediate succession stages (shrublands) were characterised by higher woody plant species diversity and spatial heterogeneity, and higher grass biomass compared to the other succession stages. Therefore, it could be hypothesised that, in intermediate succession stages, the observed higher plant taxonomic and functional diversity was associated with an increase in the exploration of belowground resources and niches (e.g. high investment in fine roots for maximization of resource





**Fig. 3.** Proportional SOC stock change between forest and previous pasture for topsoil, subsoil and the whole soil profile in the Alps (filled bars) and Apennines (empty bars). Bars represent mean values, while the error bars represent the standard deviation. Letters indicate significant or not significant differences among the two sites groups ( $P = 0.05$ ).

acquisition and colonization of deeper layers of soil profile), facilitating the organic C accumulation in the soil compartment.

The reduction of the SOC stocks between shrubland [T2] and the establishment of young forests [T4] is more difficult to explain. Other possible causes, not (or only indirectly) linked to the woody encroachment process, were considered as potential explanations, in particular: 1) the older succession stages of selected chronosequences (T4 and T5) are located in areas with steeper slope, lower accessibility and lower soil fertility with respect to younger succession stages; 2) SOC leaching and

3) a SOC mineralisation process potentially promoted in some succession stages with respect to others. However:

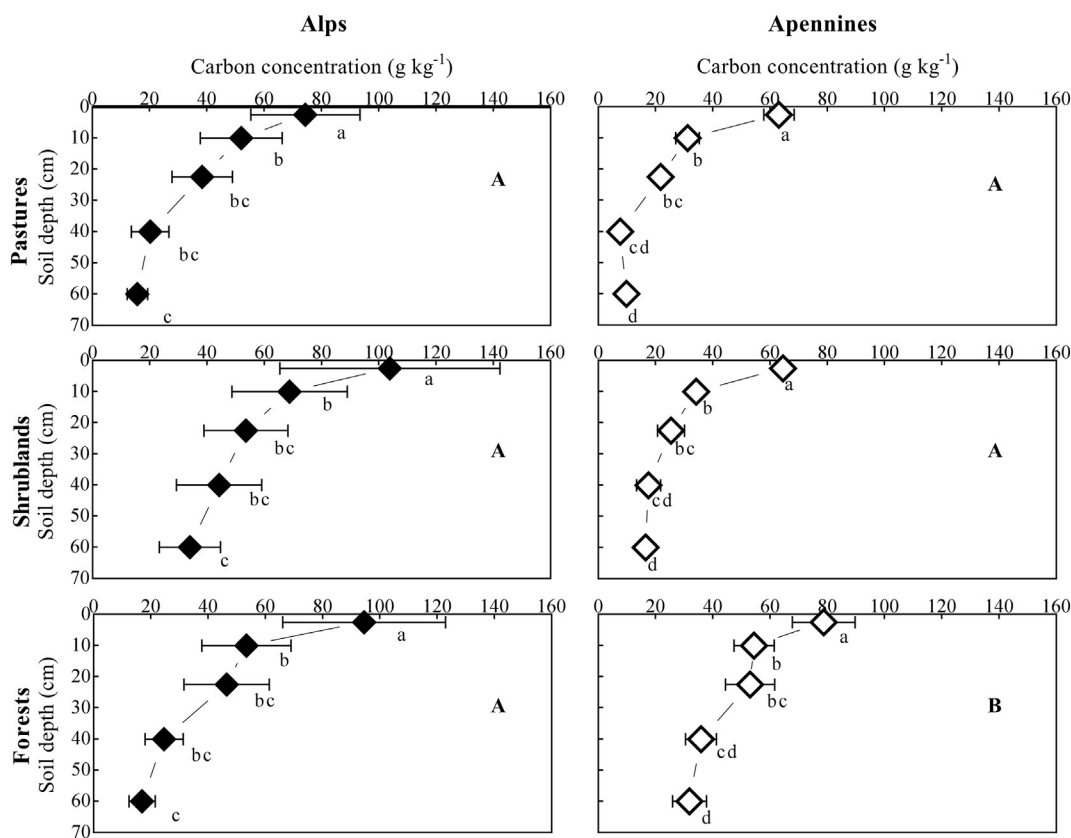
1) The linear mixed-effect model did not identify slope position and steepness as significant parameters in explaining SOC stock changes, suggesting that their variability in each chronosequence did not significantly affect soil erosion/deposition;

2) SOC concentration gradually decreased with soil depth similarly in all the succession stage of each chronosequence, suggesting no evidence of more intense leaching processes in one of them. Moreover, according to theory, we hypothesised that the progressive canopy closure woody encroachment processes facilitate SOC accumulation reducing both horizontal soil erosion and vertical  $C_{org}$  translocation and;

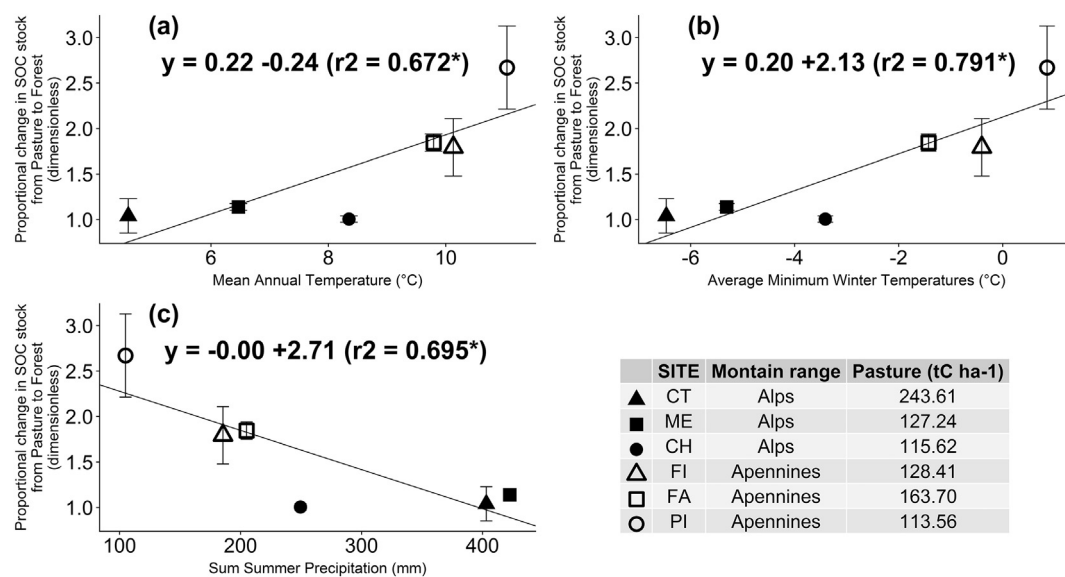
3) No consistent correlations were observed between SOC stock and microbial biomass, a microbial synthetic enzymatic index and soil heterotrophic respiration in the 0–5 and 5–15 cm soil depth intervals of all pasture, shrubland and mature forest succession stages (Pellis et al., in preparation).

Though it was not possible to definitively exclude the contribution of these causes and the role of their interactions, the main likely cause of the SOC stock decrease during the intermediate phase of the process was the change in vegetation type, from a taxonomic and functionally diverse intermediate state to a tree-dominated ecosystem. Therefore, young forest succession stages, where slow accumulation of recalcitrant compounds derived from woody vegetation (above- and below-ground litter) occurs, were arguably unable to compensate for the drop of the input of more labile compounds like herbaceous litter (Thuille and Schulze, 2006; Poeplau et al., 2011) and manure. In addition, according to Muys et al. (1992) and Poeplau et al. (2011), trees coverage likely suppresses bioturbation activity, especially under conifer stands.

All sites were characterised by similar SOC stock patterns over time during the young forest succession stages of woody encroachment.



**Fig. 4.** C concentration (mean  $\pm$  se) along the soil profile in the three most representative succession stages of the process. The panels are grouped in Alps (on the left) and Apennines (on the right). Lower case letters indicates significant ( $P < 0.05$ ) differences among depth layer C concentration in each panel, while capital case letters indicates significant ( $P < 0.05$ ) differences among succession stages in each group of sites.



**Fig. 5.** Forest to pasture SOC stock changes as a function of sites' mean annual temperatures – MAT – (a) the average of minimum temperature of winter period –  $T_{min,w}$  (b), the sum of summer months' precipitation – Ps – (c). Sites symbols (mean  $\pm$  sd) are divided in filled (presence of conifers, Alps) and empty ones (absence of conifers, Apennines).

However, marked differences between Alps and Apennines sites were observed in the mature forest succession stages [T5]. While in the sites located in the Alps woody encroachment did not affect soil carbon stocks, in those located in the Apennines soil carbon stocks increased. These differences may be explained by both vegetation type and climate. Indeed, temperature does not only affect the dead organic matter degradation rate (its mineralisation is exponentially correlated with temperature (Jenkinson et al., 1991)), but also tree plant species composition: conifers presence (in the Alps) and their absence (in the Apennines) (Paul et al., 2002). Moreover, these taxa differ not only in root depth distribution (generally deeper in broadleaves than in conifers) but also in the absence of herbaceous vegetation in conifer forests (Vesterdal et al., 2002; Pérez-Cruzado et al., 2011; Poeplau and Don, 2013), in the different litter quality (C/N conifers > C/N broadleaves) (Rey and Jarvis, 2006) and, therefore, in litter decomposability rate (Vesterdal et al., 2002; Vesterdal et al., 2008; Pérez-Cruzado et al., 2011; Poeplau and Don, 2013). Furthermore, the higher amount of recalcitrant chemical compounds in the conifers forest floor (high C/N, phenols, lignins, suberins, etc.) tends to reduce litter palatability and soil pH, which in turn, negatively affect both nutrient availability for microbes (Miles, 1985; Lucas-Borja et al., 2010) and earthworm activity (Muys et al., 1992).

On average, SOC stocks measured in the whole soil profile ranged between 136 and 249 Mg C ha<sup>-1</sup>. This is somewhat consistent with the slightly lower values (101–140 Mg C ha<sup>-1</sup>) observed by Hiltbrunner et al. (2013) in the 0–80 cm depth layer. Comparisons with other studies in the Alps region (Thuille et al., 2000; Thuille and Schulze, 2006; Risch et al., 2008; Guidi et al., 2014a) are less reliable because in these studies SOC stock was estimated in a shallower soil portion. No comparison is possible instead for the Apennines mountain ridge, due to the lack of previous studies in that region.

## 5.2. Changes in SOC through the soil profile

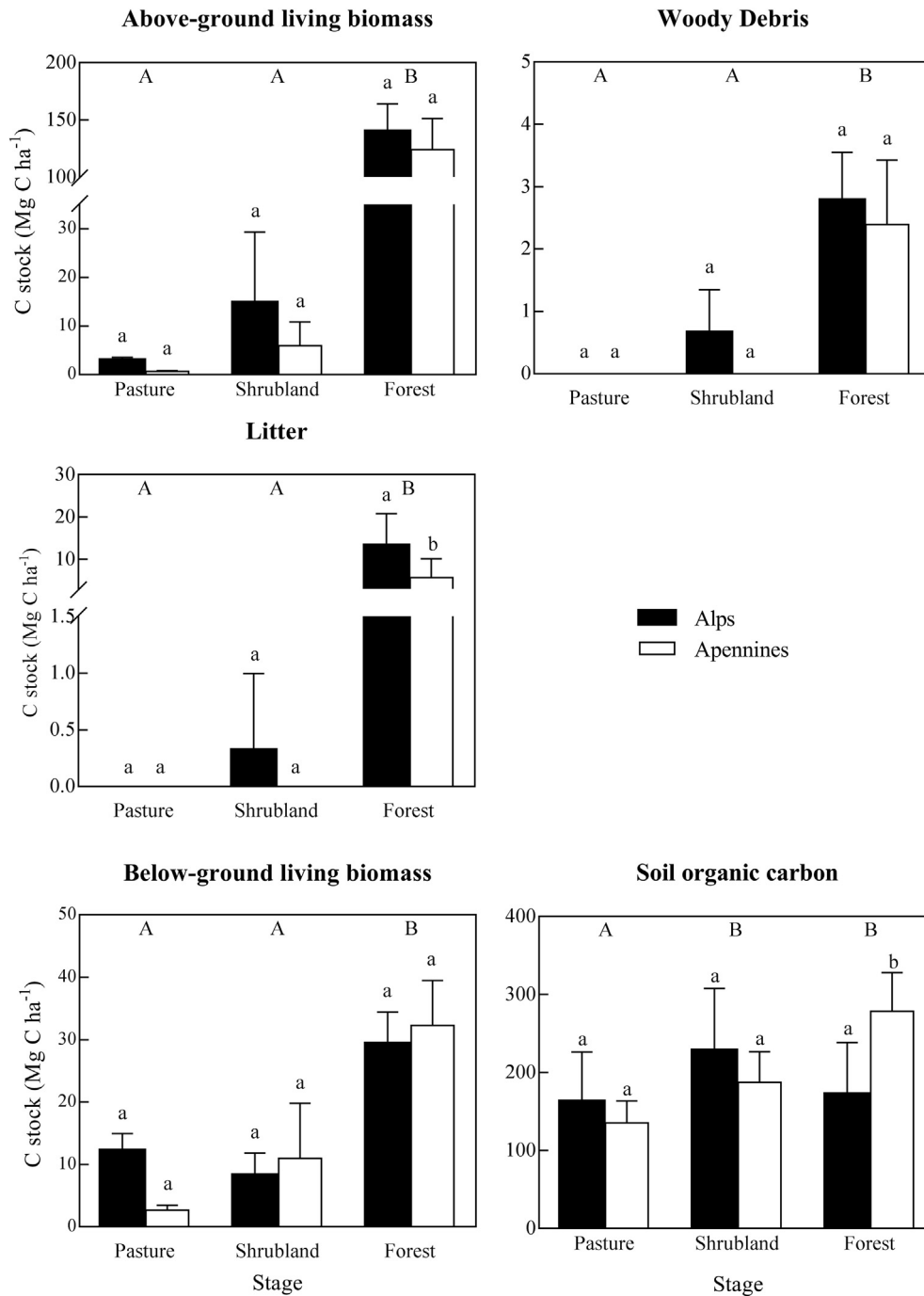
Generally, studies that deal with LUC effects on SOC stock focus only on the topsoil (e.g. Harrison et al., 2011; Hiltbrunner et al., 2013; Poeplau and Don, 2013) because it is subjected to a more important C input and a more rapid SOC turnover (Rey et al., 2008; Conant et al., 2001) with respect to the subsoil, as well as simply being easier to sample. However, our results showed that the subsoil is an important reservoir of organic C in all the considered succession stages (average of 38%). This result is supported by the marked capacity of the subsoil to

store C as observed by several authors (e.g. Batjes, 1996; Jobbágy and Jackson, 2000; Lorenz and Lal, 2005; Don et al., 2007; Poeplau et al., 2011; Poeplau and Don, 2013). In agreement with other studies (Poeplau et al., 2011), changes of SOC stocks over time identified by the linear mixed-effect models occurred both in topsoil and subsoil compartments (see Tables S5 and S6).

The SOC stock increments observed in the subsoil were in line with the main theories regarding SOC inputs in deep soil layers. Indeed, on the one hand, maximum values were observed under shrubs and mature broadleaves, plant functional types that are characterised by deeper root systems than those of grasses and conifers (Jackson et al., 1996; Jobbágy and Jackson, 2000), and that, therefore, directly release exudates and dead organic matter (root turnover) in the subsoil (Jobbágy and Jackson, 2000; Poeplau and Don, 2013). On the other hand, the higher subsoil SOC stock observed in broadleaf mature forests compared to conifer mature forests can also be explained by higher soil pH, higher litter palatability and higher grass biomass in the former. In addition, low pH and low litter palatability typical of needle-leaf forests strongly limit the abundance and activity of earthworms (Muys et al., 1992), main macroinvertebrates responsible for the SOC translocation from upper to lower soil layers (Seeber et al., 2005; Poeplau and Don, 2013).

## 5.3. The role of climate on the effect of woody encroachment on SOC stocks

The role of forest type (i.e. conifers and broadleaves) on SOC stock changes is also inevitably linked to site climate parameters. In this study, we observed that SOC stock proportional changes (ratios) have a good correlation with i) mean annual temperature (MAT), ii) the average of the minimum winter temperatures ( $T_{min,w}$ ) and iii) the sum of summer precipitations (Ps); note however that these three factor are highly correlated. No significant correlation between mature forest to pasture SOC stock ratio (or difference) and mean annual precipitation (MAP) was found. A possible justification is the fact that the MAP range considered in this study (957–1670 mm yr<sup>-1</sup>) was smaller than that considered in others (200–1100 mm yr<sup>-1</sup> in Jackson et al., 2002, and 650–2415 mm yr<sup>-1</sup> in Alberti et al., 2011) and we did not study extremely dry or extremely wet sites. Instead, the latitudinal and elevation gradient of the sites along the Italian peninsula allowed a large range of temperature values (4.6 °C and 11 °C). In addition, the significant role of both  $T_{min,w}$  and Ps indicate that, in the studied areas specific climatic condition of summer and winter periods can have a considerable role



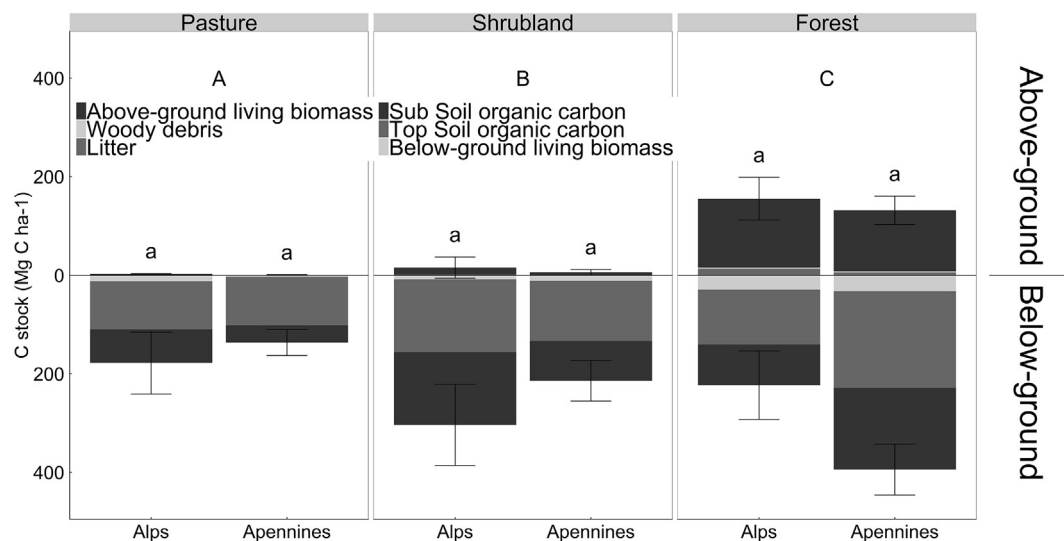
**Fig. 6.** C stocks amounts (mean  $\pm$  sd) in all the ecosystem pools, separately. Data refers to the three main woody encroachments' stages only (pastures, shrublands and forest). Results were organised by sites' groups: Alps (black bars) and Apennines (empty bars). For each pool, C stocks were labelled to highlight significant differences ( $P < 0.05$ ) among between group of sites (lower case letters) or among succession stages (capital letters).

in the SOC stock changes over time, especially in the occurrence of extreme frost and drought events as demonstrated by Frank et al. (2015).

Our results, differing from those of Jackson et al. (2002) and Alberti et al. (2011) who observed that SOC changes from grazing land to succession stage are negatively correlated with MAP, are more in line with several studies in different parts of the world where SOC stock changes due to secondary succession processes do not depend on MAP (e.g. Pinno and Wilson, 2011; Fonseca et al., 2011; Chiti et al., 2016). In addition, the role of temperature has also been recognised as an important determinant of SOC stock changes along secondary successions by Jobbágy and Jackson (2000), Alberti et al. (2011), Guidi et al. (2014a),

Thuille and Schulze (2006), but all these studies still consider the MAP as the dominant factor. However, in accordance with Poeplau et al. (2011) and La Mantia et al. (2013), our results indicate a more substantial role of temperature with respect to precipitation in affecting SOC changes along woody encroachment process.

Chianocco site (CH) does not fit with the linear regression in any of the Fig. 5 plots, maintaining a SOC stock proportional change (ratio) close to 1, similar to that of the other conifer dominated and sites (CT and ME) located in the Alps. Therefore, two groups of sites can be identified: conifer dominated sites with a negligible effect on SOC stock change, and broadleaves dominated sites, with an evident positive



**Fig. 7.** Ecosystem C stocks (mean  $\pm$  sd) along the three main succession stages (figure panels) of the woody encroachment process in both Alps and Apennines. All the bars are divided by C pools. Each of them is represented by a specific colour according to the legend. There is only one legend that refers to all the panels of the figure. It is subdivided in two columns: the first one describes the above-ground C pools (upper part of the figure), the second column describes the below-ground C pools (lower part of the figure). Capital letters refer to significant differences ( $P < 0.05$ ) in the C stock among succession stages (they are not panel labels). Lower case letters indicate that there are no significant differences in ecosystem C stocks between correspondent succession stages in Alps and Apennines.

effect on it. This outcome confirms the results of Guo and Gifford (2002), who pointed out that conifer and broadleaf forest plantations over pasture can have different effect on SOC stock changes.

On one side, these results are based on only 6 sites along Italian peninsula and, therefore, generalisations should be made with caution.

On the other side, these results could be particularly helpful for future researches, because:

- They are based on data which were collected following standardised methodological procedures. Therefore, their analyses can lead to more robust sites' comparison with respect to that based on review studies.
- They specifically refer to woody encroachment, a much less studied process than afforestation/plantation, which could substantially affect SOC stock changes because of the site preparation.

#### 5.4. Effect of woody encroachment on ecosystem C stocks

The increment in AGB observed in this study with woody encroachment is in agreement with other studies (see Thuille et al., 2000; Thuille and Schulze, 2006; Alberti et al., 2008; Risch et al., 2008; Fonseca et al., 2011; Hiltbrunner et al., 2013; Guidi et al., 2014a). As the BGB was estimated as a function of AGB by means of the root-to-shoot ratio (R/S ratio), it followed the same positive trend over time as described by Pinno and Wilson (2011).

Dead organic matter followed the same increment along the process as that observed for AGB and BGB. At the end of the process, it reached a maximum C stock similar to that estimated by Alberti et al. (2008) for a 75-year-old forest succession stage studied in the Eastern Prealps. Similar results have also been reported in other studies carried out in the Alps (see Thuille et al., 2000; Thuille and Schulze, 2006; Risch et al., 2008; Hiltbrunner et al., 2013). This trend could be explained not only by the increment in the dead organic matter input (Brown and Lugo, 1990) and the reduction in the dead organic matter degradability previously discussed, but also by the reduction in summer temperatures and soil moisture with forest canopy closure (Thuille and Schulze, 2006). Indeed, soil temperature and moisture are known to strongly affect litter and soil organic matter decomposition (Trofymow et al., 2002; Zhang et al., 2008; Rey et al., 2008).

The differences in litter accumulation between the sites in the Alps and Apennines were mainly linked to the diversity in litter quality between broadleaves and conifers.

At ecosystem level, the living biomass acted as a significant C sink mostly in the last part of the process, while in the initial succession stages its contribution was limited. Similar results have been observed by Thuille et al. (2000), Risch et al. (2008), Alberti et al. (2008), Hiltbrunner et al. (2013) and Guidi et al. (2014a). On the other hand, the main C pool was always the soil, which never decreased below the 40% threshold. In the mature forest succession stage, this percentage was significantly higher than the 20–25% reported by Thuille and Schulze (2006) and Guidi et al. (2014a). This may be explained by both the relatively high SOC content of our selected sites and by the deeper soil profile considered for the SOC stock estimation.

Jackson et al. (2002) suggested that, at ecosystem level, a possible SOC loss could be large enough to offset the increase in plant biomass. However, all the study areas considered here showed an increase in ecosystem C stocks without reduction of the SOC stock along the woody encroachment process. Therefore, it is possible to conclude that in the Italian peninsula, land-use changes from pastures/grasslands to forest act as a sink for organic carbon. For this reason, the process of woody encroachment could represent an important strategy to reduce the amount of CO<sub>2</sub> in the atmosphere.

## 6. Conclusions

Woody encroachment resulted in a significant increase in soil carbon stocks in the first phase of the successional process (from pasture to shrubland) in all mountainous sites. However, in the second phase SOC stocks changes significantly diverged between sites: while SOC stocks decreased in mature conifer forest, they increased in mature broadleaf forests with increasing stand age. We attribute these differences in the response of plant functional types to differences in root system development and litter quality. As a consequence, we propose that the default period of 20 years suggested by the IPCC for reaching SOC stock equilibrium after land use change should be revised. Our results show that, in the Alps sites characterised by conifer forest, a steady state is reached approximately 28 years after the woody encroachment's start. Other studies in the Alps report even higher values, though in relation to different vegetation types: 35 years for



Guidi et al. (2014a), who after that did not detect significant SOC stock differences with mature forest stages (150 years old), despite registering a slight decrement; and no <55 years for Alberti et al. (2008), who noticed a significant SOC stock increase between then and the 70 years old forest. However, it is worth mentioning that these authors considered only the topsoil compartment and mix and broadleaf forest types, respectively. There are no other studies in the Apennines, but our results there show that, even after 70 years, a steady state might have not been reached for broadleaf forests. Therefore, a single criterion for the time required to reach the SOC stock steady state might be difficult to formulate, as values appear to depend on both climate conditions and forest vegetation types.

In addition, we observed that the subsoil was an important C reservoir, storing 38% of the whole SOC stock and exceeding the amount of carbon stored in the topsoil in some cases. Moreover, the SOC stock changes in the subsoil were, in general and unexpectedly, larger than those of the topsoil. Therefore, this study highlights the importance of considering the entire soil profile, and not only the topsoil compartment, for an accurate estimation of the impact of secondary successions on terrestrial ecosystem C stocks. Furthermore, this finding suggests that current estimates could be clearly underestimated since only the topsoil is considered.

Even if previous studies identify MAP as the main driving factor controlling SOC stock changes along woody encroachment, we observed that MAT, the average of minimum winter temperatures ( $T_{min,w}$ ) and the sum of summer precipitations (Ps) were the best predictors. Because of the widespread woody encroachment worldwide, these results will be particularly helpful in refining the estimation of the ecosystems C storing capacity under pressing global warming scenarios. However, future studies should be focussed in the Mediterranean, a region where both high temperature increment and precipitation reduction are expected (Giorgi and Lionello, 2008). Indeed, only a few studies on this topic are available for this region.

Finally, our results revealed that the woody encroachment process over abandoned grazing land in the Italian mountain territory always acted as a C sink. Nevertheless, between the stable conditions of both grazing land and mature forest, there is a transient phase with interesting dynamics in terms of SOC stocks, living biomass and spatial heterogeneity of the vegetation, which is as yet poorly understood. These results should be taken into account when refining the quantification of the land-use change C sink/source effects, as requested by the United Nations Framework Convention on Climate Change.

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